

AMENDMENT OF THE SPECIFICATION

Please amend the specification as follows:

On page 4, replace the first paragraph (lines 1 and 2) with the following paragraph:

In an exemplified embodiment, the co-culturing is in a medium containing N2 SUPPLEMENT®.

On page 7, replace the first paragraph (lines 1-6) with the following paragraph:

“Neuronal differentiation factors” are chemical or biological factors that induce differentiation of stem cells into cells of the neuronal lineage. Neuronal differentiation factors of the invention include, but are not limited to, basic fibroblast growth factor, fibroblast growth factor-8, brain-derived neurotrophic factor, Sonic Hedgehog, N2 SUPPLEMENT®, and combinations thereof that are capable of modulating neuronal differentiation of stem cells in culture.

On page 7, replace the fourth paragraph (lines 17-27) with the following paragraph:

Culture methods of the invention comprise an ordered addition of neuronal differentiation factors, wherein there is a first addition of basic fibroblast growth factor (Abraham, J. A. , 1986); a second addition of fibroblast growth factor 8 (Gemel, J. , 1996; Yoshiura, K. , 1997) and Sonic Hedgehog (Marigo, V. , 1995); a third addition of brain-derived neurotrophic factor (Maisonpierre, P. C., 1991) followed by co-culture with fetal brain astrocytes. Co-culturing can be performed in a medium having a supplement comprising insulin, transferrin, selenite, putrescine and progesterone. In an exemplified embodiment, the co-culturing is in a medium containing N2 SUPPLEMENT®, available from Gibco (Catalog No. 17502048, containing recombinant human insulin, human transferrin (iron-saturated), sodium selenite, putrescine and progesterone in Phosphate Buffered Saline).

On page 11, replace the first paragraph (lines 1-9) with the following paragraph:

Stem cells can be maintained and allowed to expand in culture medium (i. e., an “initial culture”) that is well established in the art and commercially available from the American Type Culture Collection (ATCC). Such media include, but are not limited to, DULBECCO’S MODIFIED EAGLE’S MEDIUM® (DMEM), DMEM F12 MEDIUM®, EAGLE’S MINIMUM ESSENTIAL MEDIUM®, F-12K MEDIUM®, ISOCOVE’S MODIFIED DULBECCO’S MEDIUM®, RPMI-1640 MEDIUM®. It is within the skill of one in the art to modify or modulate concentrations of media and media supplements as necessary for the stem cells used. It will also be apparent that many media are available as a low-glucose formulation, with or without sodium pyruvate.

On page 11, replace the last paragraph (starting at line 31; page 23, lines 1-11) with the following paragraph:

Additional supplements can also be used to supply the stem cells with the necessary trace elements for optimal growth and expansion. Such supplements include insulin, transferrin, sodium selenite and combinations thereof. These components can be included in a salt solution such as, but not limited to HANK’S BALANCED SALT SOLUTION® (HBSS), EARLE’S SALT SOLUTION®, antioxidant supplements, MCDB-201® supplements, phosphate buffered saline (PBS), ascorbic acid and ascorbic acid-2-phosphate, as well as additional amino acids. Many cell culture media already contain amino acids, however some require supplementation prior to culturing cells. Such amino acids include, but are not limited to, L-alanine, L- arginine, L-aspartic acid, L-asparagine, L-cysteine, L-cystine, L-glutamic acid, L- glutamine, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. It is well within the skill of one in the art to determine the proper concentrations of these supplements.

On page 12, replace the first full paragraph (lines 12-23) with the following:

Antibiotics are also typically used in cell culture to mitigate bacterial, mycoplasmal, and fungal contamination. Typically, antibiotics or anti-mycotic compounds used are mixtures of penicillin/streptomycin, but can also include, but are not limited to amphotericin (FUNGIZONE®), ampicillin, gentamicin, bleomycin, hygromycin, kanamycin, mitomycin, mycophenolic acid, nalidixic acid, neomycin, nystatin, paromomycin, polymyxin, puromycin, rifampicin, spectinomycin, tetracycline, tylosin, and zeocin. Antibiotic and antimycotic additives can be of some concern, depending on the type of work being performed. One possible situation that can arise is an antibiotic-containing media wherein bacteria are still present in the culture, but the action of the antibiotic performs a bacteriostatic rather than bacteriocidal mechanism. Also, antibiotics can interfere with the metabolism of some cell types.

Replace Table 1 on page 20 with the following:

Table 1: Primers used for Q-RT-PCR

Gene	Forward	Reverse	Size	
(SEQ ID NO: 1)	Sox-1	AAGATGCACAACTGGAGATCAG	TGTAATGGGTGTGTCCTCAT	(SEQ ID NO: 17)
(SEQ ID NO: 2)	Otx-2	CCATGACCTATCTCAGGCTTCAGG	GAAGCTOCATATGCTGGGTGQAAAG	(SEQ ID NO: 18)
(SEQ ID NO: 3)	Otx-1	AGGCGCTGTTGGCAAAGA	CTCTCTGGGCGCATGAAGAT	(SEQ ID NO: 19)
(SEQ ID NO: 4)	Pax-2	CCAGGCATCAGAGCACATCA	CGTCTGTGTGCTGACACATT	(SEQ ID NO: 20)
(SEQ ID NO: 5)	Pax-5	AAACGCAAGAGGGATGAAGGT	AACAGGTCTGCTGCTGCT	(SEQ ID NO: 21)
(SEQ ID NO: 6)	Pax-3	TGTGTGGCACTGGAGTTCA	CACCTCAGGAACAGAGTGACTT	(SEQ ID NO: 22)
(SEQ ID NO: 7)	CR4	GAGGAAATGTACCGTCTGATGCT	TCTTGACCATCATCTTCTCCAGATC	(SEQ ID NO: 23)
(SEQ ID NO: 8)	Nurr-1	TGAAGAGAGCGGAGAGGAGATC	TCTGGAGTTAAGAAATGGGAGCTG	(SEQ ID NO: 24)
(SEQ ID NO: 9)	Nestin	GAGAAGACAGTGAGGCAGATGAGTTA	GCCTCTGTCTCCAGCTTGCT	(SEQ ID NO: 25)
(SEQ ID NO: 10)	GFAP	GAGGAGTGGTATGGGTCTAAGTTTG	GGCGCTCTAGGGAGCTGGT	(SEQ ID NO: 26)
(SEQ ID NO: 11)	MBP	GTGCAGCTTGTTCGACTGCG	ATGCTCTCTGGCTGCTTGCC	(SEQ ID NO: 27)
(SEQ ID NO: 12)	GABA	AGGTTGACCGTGGAGAGCTGAAT	TGGGCGAGGCATGGGC	(SEQ ID NO: 28)
(SEQ ID NO: 13)	DAT	GCAATCATCAACCACTCCATTA	ATGGGCACATTTGCTTCTG	(SEQ ID NO: 29)
(SEQ ID NO: 14)	TH	AGTTCTCCAGGACATTTGGACTT	ACACAGGCCAAACTCCACAGT	(SEQ ID NO: 30)
(SEQ ID NO: 15)	TyH	GGATGGAGTCTGATGTCAACAA	TGACGTTTCTCAGGCATTAAAGC	(SEQ ID NO: 31)
(SEQ ID NO: 16)	DBH	TTCGAATGTGCAGCTGAGTC	GGTGCACCTTCTGCTGTCAGT	(SEQ ID NO: 32)